

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

CHEMISTRY AND THE ENVIRONMENT DIVISION
COMMISSION ON OILS, FATS, AND DERIVATIVES*†

DETERMINATION OF POLAR COMPOUNDS, POLYMERIZED AND OXIDIZED TRIACYLGLYCEROLS, AND DIACYLGLYCEROLS IN OILS AND FATS

Results of Collaborative Studies and the Standardized Method

(Technical Report)

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Determination of polar compounds, polymerized and oxidized triacylglycerols, and diacylglycerols in oils and fats (Technical Report)

Abstract: A description is given of the development by collaborative study of two standardized methods for the determination of polar compounds in oils and fats by adsorption chromatography using silica minicolumns, and for quantification of polymerized triacylglycerols, oxidized triacylglycerols, and diacylglycerols in polar compounds by high-performance size-exclusion chromatography. The first procedure is sensitive, allowing savings in time, solvents, and reagents as compared to the previous determination (Standard Method 2.507), while the second is very rapid, giving a detailed information on the main groups of compounds in fats and oils associated with hydrolysis, oxidation, and thermal polymerization. Both methods are useful for the analysis of used frying fats as well as for the analysis of virgin or refined oils.

INTRODUCTION

Determination of polar compounds in head-abused oils and fats is a well-accepted method due to its accuracy and reproducibility [1]. The level of polar compounds is a good indicator of the quality of used frying fats and oils, giving information of the total amount of newly formed compounds having higher polarity than that of triacylglycerols and being the basis of the present recommendations and regulations limiting degradation of used frying fats for human consumption [2]. Complementary determination of polymerized triacylglycerols in fats and oils by exclusion chromatography allows quantification of the main groups of compounds formed during the frying process [3].

In the last decade, two interesting analytical approaches for used frying fat evaluation have been suggested: 1) determination of polar compounds by using minicolumns [4] with the advantages of reducing time, silica, and then solvents with respect to the standard method and 2) application of exclusion chromatography to the polar compound fraction as a means of increasing knowledge on hydrolytic, oxidative, and polymerization compounds [5,6].

Initially, the Commission considered studying the application of exclusion chromatography to the polar fraction obtained by the IUPAC Standard Method 2.507 for quantification of oxidized triacylglycerols and then, in a second step, to combine both new possibilities to quantify polar compounds by silica minicolumns and the main groups included in the polar fraction, for example, polymerized triacylglycerols, oxidized triacylglycerols, and diacylglycerols, by high-performance size-exclusion chromatography (HPSEC).

METHODS OF ANALYSIS

Figure 1 shows the analytical procedure combining the isolation of polar compounds by adsorption chromatography and the later separation of polar compounds by HPSEC. In the first step, nonpolar fraction and polar compounds are eluted with solvents of different polarity. Even if the fraction of polar compounds is low, four groups of compounds differing in molecular weight can be well resolved, as observed in the figure: polymerized triacylglycerols (PTG), oxidized triacylglycerols (OTG), diacylglycerols (DG), and fatty acids (FA). This last peak also includes polar unsaponifiable materials of molecular weight similar to that of free fatty acids.

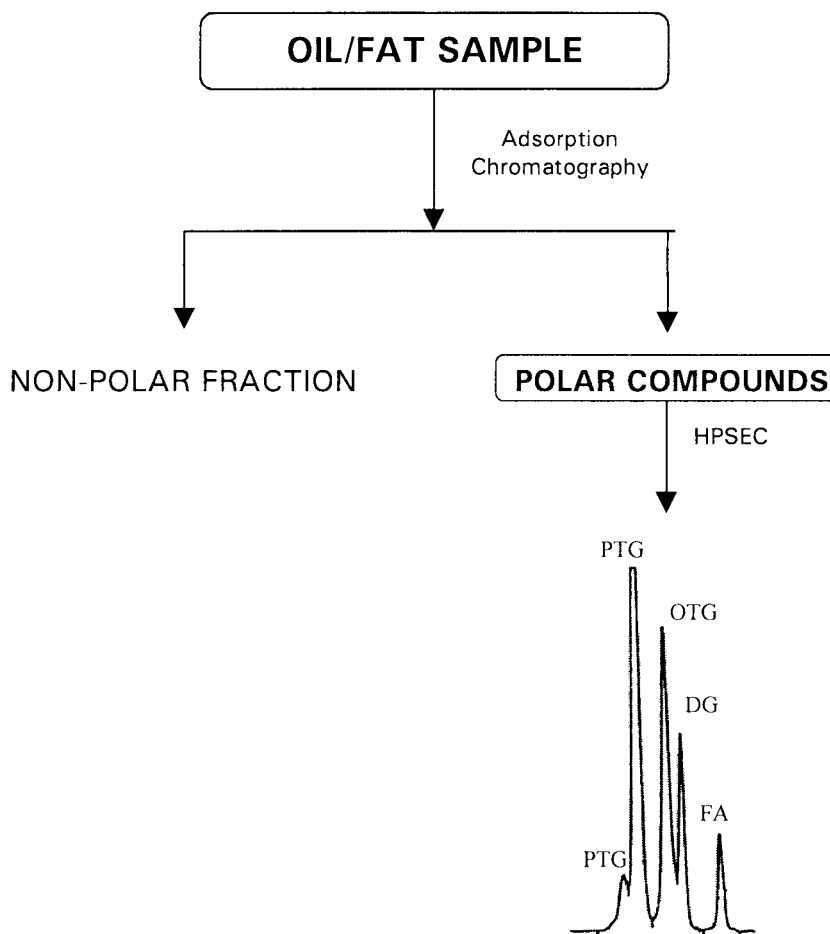


Fig. 1 Analytical procedure for determination of total polar compounds and their distribution. Abbreviations: HPSEC, high-performance size-exclusion chromatography; PTG, polymerized triacylglycerols; OTG, oxidized triacylglycerols; DG, diacylglycerols; FA, fatty acids.

Collaborative studies

In the first collaborative study (1993–94), isolation of polar compounds was carried out following the standard method 2.507 for determination of polar compounds in frying fats while in the second one (1995–96) silica minicolumns were used as detailed in the new method for polar compound determination (Appendix 1). In both ring tests, the isolated polar fraction was evaluated by means of HPSEC under the conditions specified in the proposed new method for polar compound distribution (Appendix 2).

First study

Samples were distributed to 18 participating laboratories in November 1993. Thirteen laboratories transmitted their results before 20 May 1994. Samples consisted of blind duplicates of three oils: 1) refined high oleic sunflower oil with very low level of polar compounds, 2) refined palm oil with very low level of oxidized triacylglycerols, and 3) used frying oil with intermediate level of both polar compounds and oxidized triacylglycerols.

Two laboratories sent quantitative results on polar compounds but HPSEC was not applied to polar compounds. Two sets of results using an evaporative light-scattering detector instead of refractive index detector for HPSEC analysis were received.

Second study

Samples with different levels of polar compounds were distributed to 11 participants in December, 1995. Ten laboratories sent their results before 15 June 1996. Samples consisted of blind duplicates of five oils: 1) refined high oleic sunflower oil with very low level of polar compounds, 2) refined palm oil with very low level of oxidized triacylglycerols, 3) olive oil used in discontinuous frying, 4) a mixture of used frying high oleic sunflower oil and palm olein, and 5) high oleic sunflower oil rejected from domestic frying. Only one set of results were obtained using an evaporative light-scattering detector instead of the recommended refractive index detector.

RESULTS

For statistical analysis, the International Standard ISO 5725 was followed. Results of the statistical analysis obtained for determination of polar compounds and oxidized triacylglycerols in the first ring test are summarized in Tables 1 and 2, respectively. Parallel results for the second ring test by using

Table 1 1st collaborative study: Statistical analysis results for determination of polar compounds*.

Sample	1	2	3
Number of laboratories	13	13	13
Number of accepted result sets	11	11	13
Mean mass fraction	2.8%	8.4%	13.7%
Repeatability			
Standard deviation S_r	0.27%	0.32%	0.41%
Repeatability r	0.76%	0.90%	1.15%
Coefficient of variation CV_r	9.6%	3.8%	3.0%
Reproducibility			
Standard deviation S_R	0.65%	0.70%	1.72%
Reproducibility R	1.83%	1.97%	4.85%
Coefficient of variation CV_R	23.2%	8.3%	12.5%

* For the determination of polar compounds, IUPAC Standard Method 2.507 was applied

Table 2 1st collaborative study: Statistical analysis of results for determination of oxidized triacylglycerols

Sample	1	2	3
Number of laboratories	11	11	11
Number of accepted result sets	9	9	11
Mean mass fraction	0.8%	0.4%	5.5%
Repeatability			
Standard deviation S_r	0.07%	0.03%	0.21%
Repeatability r	0.20%	0.08%	0.59%
Coefficient of variation CV_r	8.2%	8.5%	3.8%
Reproducibility			
Standard deviation S_R	0.30%	0.12%	0.80%
Reproducibility R	0.85%	0.34%	2.26%
Coefficient of variation CV_R	35.9%	30.5 %	14.6%

Table 3 2nd collaborative study: Statistical analysis of results for determination of polar compounds.

Sample	1	2	3	4	5
Number of laboratories	10	10	10	10	10
Number of accepted result sets	9	9	10	8	10
Mean mass fraction	3.2%	8.7%	13.4%	17.0%	29.3%
Repeatability					
Standard deviation S_r	0.32%	0.77%	1.25%	0.98%	0.59%
Repeatability r	0.89%	2.16%	3.50%	2.74%	1.65%
Coefficient of variation CV_r	10.2%	8.9%	9.4%	5.8%	2.0%
Reproducibility					
Standard deviation S_R	1.28%	1.16%	2.31%	1.16%	2.38%
Reproducibility R	3.58%	3.25%	6.47%	3.25%	6.66%
Coefficient of variation CV_R	40.5%	13.2%	17.3%	6.6%	8.1%

Table 4 2nd collaborative study: Statistical analysis of results for determination of polymerized triacylglycerols.

Sample	1	2	3	4	5
Number of laboratories	10	10	10	10	10
Number of accepted result sets	10	9	9	10	10
Mean mass fraction	0.6%	0.6%	5.7%	6.9%	15.9%
Repeatability					
Standard deviation S_r	0.08%	0.09%	0.37%	1.06%	0.36%
Repeatability r	0.22%	0.25%	1.04%	2.97%	1.01%
Coefficient of variation CV_r	13.3%	15.0%	6.5%	15.4%	2.3%
Reproducibility					
Standard deviation S_R	0.35%	1.16%	0.8%	1.15%	1.35%
Reproducibility R	0.98%	3.25%	2.46%	3.22%	3.78%
Coefficient of variation CV_R	58.3%	56.7%	15.5%	16.7%	8.5%

Table 5 2nd collaborative study: Statistical analysis of results for determination of oxidized triacylglycerols.

Sample	1	2	3	4	5
Number of laboratories	10	10	10	10	10
Number of accepted result sets	9	10	9	7	10
Mean mass fraction	1.4%	0.6%	4.3%	5.4%	10.7%
Repeatability					
Standard deviation S_r	0.15%	0.10%	0.30%	0.47%	0.39%
Repeatability r	0.42%	0.28%	0.84%	1.32%	1.09%
Coefficient of variation CV_r	11.0%	16.4%	7.0%	8.7%	3.6%
Reproducibility					
Standard deviation S_R	0.61%	0.32%	0.53%	0.57%	1.95%
Reproducibility R	1.71%	0.90%	1.48%	1.60%	5.46%
Coefficient of variation CV_R	44.4%	49.4%	12.2%	10.6%	18.2%

Table 6 2nd collaborative study: Statistical analysis of results for determination of diacylglycerols.

Sample	1	2	3	4	5
Number of laboratories	10	10	10	10	10
Number of accepted result sets	9	9	9	10	9
Mean mass fraction	1.2%	7.2%	2.5%	4.5%	2.5%
Repeatability					
Standard deviation S_r	0.08%	0.62%	0.17%	0.40%	0.07%
Repeatability r	0.22%	1.85%	0.48%	1.09%	0.20%
Coefficient of variation CV_r	7.3%	8.6%	6.9%	8.7%	2.8%
Reproducibility					
Standard deviation S_R	0.37%	0.82%	0.31%	0.92%	0.31%
Reproducibility R	1.04%	2.30%	0.87%	2.58%	0.87%
Coefficient of variation CV_R	33.6%	11.4%	12.4%	20.4%	12.4%

minicolumns for polar compound determination are shown in Tables 3 and 5. Finally, Tables 4 and 6 show statistical results for polymerized triacylglycerols and diacylglycerols, respectively.

DISCUSSION

Tables 1 and 2 showing statistical results for polar compounds in the first and Tables 3 to 6 for the second ring test, respectively, indicate that repeatability and reproducibility depended on both polar compound content and the analytical approach used for their evaluation. Thus, the higher was the polar compounds, the lower were the standard deviations. Also, as expected, the higher the initial weight sample, 1 g for the Standard Method 2.507 and 0.5 g for the minicolumn method, the lower was the standard deviation, as the method is based on a gravimetric determination. However, as it can be observed in Table 4, the coefficient of variation for repeatability by using minicolumns was as low as 2% for the sample with content of polar compounds in the order of 30 g per 100 g and 10 g per 100 g for the sample with the lowest content of polar compounds (around 3 g per 100 g).

With respect to the group of oxidized triacylglycerols quantified by HPSEC, the coefficient of variations included in Tables 2 and 5 show the dependence on three variables: the analytical method used for polar compound quantification, the level of polar compounds in the sample, and the level of oxidized triacylglycerols. Nevertheless, repeatability and reproducibility were high in all the samples considering that their content was based on the previous polar compound determination and that polar compounds are a complex group of many components containing different functional groups. Finally, repeatability and reproducibility for polymerized triacylglycerols and diacylglycerols, only quantified in the second ring test, were of the same order as those obtained for oxidized triacylglycerols.

CONCLUSION

The analytical procedure is sensitive and allows reliable determination of polar compounds in fats and oils as well as differentiation of the main groups of components included in the polar fraction, giving information on oxidation, hydrolysis, and thermal alteration.

Based on the repeatability and reproducibility of the results obtained in the second ring test, the Commission decided to adopt the method for determination of polar compounds by using minicolumns and for determination of the main groups included in the polar fraction by means of HPSEC. The texts for both standardized procedures are given in the following pages.

ACKNOWLEDGMENT

The Commission is thankful to the collaborators in Brazil, Canada, Czech Republic, Denmark, France, Greece, Hungary, Japan, The Netherlands, Spain, and Switzerland for their participation and valuable cooperation.

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APPENDIX 1. DETERMINATION OF POLAR COMPOUNDS IN OILS AND FATS (MINICOLUMN METHOD)

1. Scope

This Standard describes a method for the determination of polar compounds in frying oils and fats as well as those present in refined or virgin oils.

2. Field of application

This Standard is applicable to animal and vegetable oils and fats. The method serves to assess the deterioration of used frying fats.

3. Definition

Polar compounds are those compounds in fats which are determined by column chromatography under the conditions specified (*Note 1*).

4. Principle

Selective adsorption of polar and nonpolar lipids on a partly deactivated silica gel column. Elution of the nonpolar and polar compounds. Confirmation of the efficiency of separation by thin-layer chromatography.

5. Apparatus

- 5.1 100- and 500-mL round-bottomed flasks with ground necks
- 5.2 100-mL beakers
- 5.3 Ground-glass stoppers to fit the 500-mL flasks (5.1)
- 5.4 Chromatographic glass column, 10-mm internal diameter, 150-mm length, with stopcock (preferably in polytetrafluorethylene) with glass joint
- 5.5 100-mL dropping funnel with ground-glass joint to fit the column (5.4)

- 5.6 Glass funnel, about 8-cm diameter
- 5.7 Glass rod, about 30-cm length
- 5.8 10-mL volumetric flask
- 5.9 5-mL volumetric pipette
- 5.10 2- μ L capillary pipettes for thin-layer chromatography
- 5.11 Glass plates for thin-layer chromatography, 20 \times 20 cm, coated with silica gel (without fluorescence indicator), 0.25-mm layer thickness
- 5.12 Glass developing tank for thin-layer chromatography, with ground-glass lid
- 5.13 Spray for thin-layer chromatography
- 5.14 Porcelain dish, about 20-cm diameter
- 5.15 Oven, regulated at 103 ± 2 °C
- 5.16 Drying oven, controllable between 120 and 160 °C
- 5.17 Water bath
- 5.18 Desiccator, containing a suitable desiccant such as silica gel with moisture indicator (blue gel)
- 5.19 Apparatus for removing solvent under vacuum (e.g., rotary evaporator)
- 5.20 Shaking machine

6. Reagents

- 6.1 Light petroleum (b.p. 40–60 °C), chromatographic quality, redistilled
- 6.2 Ethanol, 95% (v/v)
- 6.3 Chloroform, pure
- 6.4 Diethyl ether, free from peroxides and residue
- 6.5 Acetic acid, 100%, analytical reagent quality
- 6.6 Elution solvent 1: mixture of light petroleum (6.1) and diethyl ether (6.4), 90/10 (v/v)
- 6.7 Elution solvent 2: diethyl ether (6.4)
- 6.8 Developing solvent: mixture of light petroleum (6.1), diethyl ether (6.4), and acetic acid (6.5), 70/40/1, (v/v)
- 6.9 Silica gel, particle size 0.063–0.200 mm (70–230 mesh), Merck N° 7734 or equivalent, adjusted to a water content of 5% (m/m) (*Note 2*)
- 6.10 Phosphomolybdic acid, analytical reagent quality, 100 g/L solution in ethanol (6.2)
- 6.11 Sea sand, purified by acid calcined
- 6.12 Cotton wool, surgical quality
- 6.13 Nitrogen, 99.0–99.8%

7. Procedure

- 7.1 Preparation of the sample
Remove visible impurities by filtration after homogenization. If water is present, use a hydrophobic filter paper.
For semiliquid and solid samples, warm to a temperature slightly above the melting point and homogenize carefully, avoid overheating.
- 7.2 Preparation of the column
Fill the column (5.4) with about 5 mL of the elution solvent 1 (6.6). Introduce a wad of cotton wool (6.12) into the lower part of the column with the aid of the glass rod (5.7) and remove air by pressing the wool.
Prepare in a beaker (5.2) a slurry of 5 g silica gel (6.9) in about 10 mL of the elution solvent 1 (6.6) and pour this slurry into the column with the aid of the funnel (5.6). To ensure complete transfer of the silica gel into the column, rinse with the elution solvent 1.

Open the stopcock and drain off the solvent into the second beaker (5.2) until the level of the elution solvents is above the silica gel level. Level the silica gel by tapping against the column.

Add about 1 g of sea sand (6.11) with the aid of the funnel. Drain off the supernatant solvent as far as the sand layer.

7.3 Column chromatography

7.3.1 Preparation of the sample solution

Weigh, to the nearest 0.001 g, 1 ± 0.1 g of the sample prepared as (7.1) into a volumetric flask (5.8). Dissolve in about 8 mL of the elution solvent 1 (6.6) while warming slightly. Allow to cool to room temperature and fill up to the mark with the elution solvent 1.

7.3.2 Separation of the nonpolar fraction

Introduce with a volumetric pipette (5.9) 5 mL of the sample (7.3.1) onto the column prepared as (7.2). Avoid disturbing the surface (*Note 3*).

Dry two 100-mL beakers (5.1) in the oven (5.15) at a temperature of 103 ± 2 °C. Allow to cool to room temperature and weigh accurately to within 0.001 g. Place one of them under the outlet of the column. Open the stopcock and let the sample solution drain off to the level of the sand layer.

Elute nonpolar fraction (fraction 1) with 60 mL of the elution solvent 1 (6.6) using a dropping funnel (5.5). Adjust the flow rate to about 1.5 mL/min.

After completion of the elution, wash any substance adhering to the outlet of the column with the elution solvent using a pipette.

7.3.3 Separation of the polar fraction

Elute the polar fraction (fraction 2) into a second 100-mL dry flask (5.1) with 50 mL of the elution solvent 2 (6.7).

Evaporate the solvent from the flasks under vacuum in a rotary evaporator (5.19) at 60 °C. Shortly before the end of the distillation, introduce nitrogen (6.12) into the system. Dry for 1 hour under vacuum. Weigh the flasks (*Note 4*).

8. Expression of results

Fraction 1 represents nonpolar components (unaltered triacylglycerols). Fraction 2 represents polar components (altered triacylglycerols and natural accompanying polar compounds) (*Note 1*).

The mass fractions of non-polar compounds (w_{np}) and the polar compounds (w_p) are given by the formula

$$w_{np} = (m_{np}/m)100\% \quad w_p = (m_p/m)100\%$$

where m_{np} is the mass (in g) of the nonpolar fraction 1, m_p is the mass (in g) of the polar fraction 2, and m is the mass (in g) of the test portion added to the column (*Note 5*).

9. Thin-layer chromatographic assessment of column efficiency

The efficiency of the fractionation can be assessed by thin-layer chromatography. For the thin-layer chromatographic investigation prepare 10% solutions of the substances in chloroform (6.3) and apply 2 μ L spots onto a plate (5.11), using a capillary pipette (5.10).

Line the developing tank (5.12) with filter paper to achieve saturation. Place the plate in the developing tank and carry out the development tank with the developing solvent (6.8). Normally, after 35 min, the solvent front ascends to a height of about 17 cm. Remove the plate and allow the solvent to evaporate.

Spray the plate with the phosphomolybdic acid solution (6.10). After evaporation of ethanol, heat the plate in the drying oven (5.16) at 120–130 °C. As an example, see Fig. 2 showing a chromatogram obtained after fractionation of a frying fat into individual fractions.

10. Notes

1. The polar compounds include polar substances such as monoacylglycerols, diacylglycerols, and free fatty acids which occur in unused fats, as well as polar transformation products formed during frying of foodstuffs and/or during heating. Nonpolar compounds are mostly unaltered triacylglycerols.
2. Place the silica gel in the porcelain dish (5.14), dry in an oven (5.16) at 160 °C for at least 4 h and cool in a desiccator to room temperature. Adjust the silica gel to a water content of 5%: e.g., weigh 125 g of silica gel and 8 g of water in 500-ml flask (5.1). Close the with a stopper (5.3) and shake mechanically with the aid of a shaking machine (5.20) or turn the round-bottomed under flask in a rotary evaporator.
3. For fats containing low amounts of polar components the amount of sample added to the column can be raised from 0.5 g up to 1 g.
4. If a rotary evaporator is not available the elution solvent can be evaporated on a steam plate under a stream of nitrogen.
5. For samples containing great amounts of polar materials, recovery of the sample may be incomplete. This is due to small amounts of highly polar materials, generally not more than 1–2 g per 100 g, which are not eluted under the conditions specified.

Annex

1. *Repeatability*: When the mean of the values obtained from two single determinations carried out in rapid succession by the same operator, using the same apparatus under the same conditions for the analysis of the same laboratory sample, lies within the range of the mean values cited in the table below, the difference between the two values obtained should not be greater than the repeatability limit (r), which can generally be deduced by linear interpolation from the values in the table below.

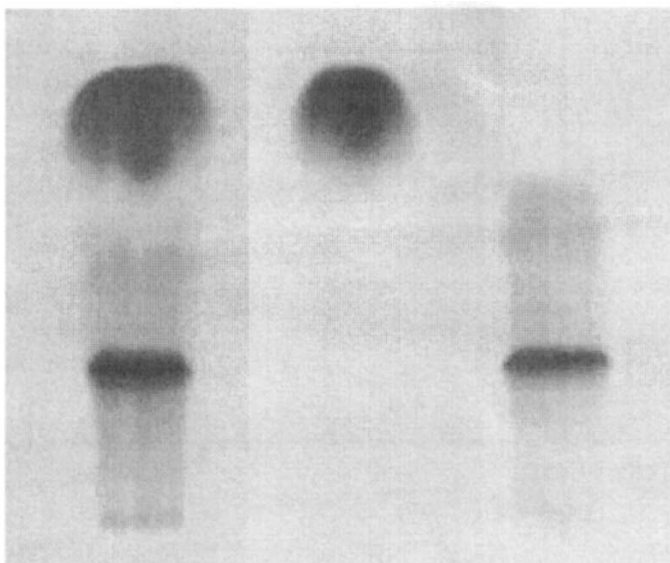


Fig. 2 Efficiency of the fractionation. 1: total sample; 2: nonpolar fraction; 3: polar compounds.

2. *Reproducibility*: When the values for the final result, obtained by operators in different laboratories using different apparatus under the same conditions for the analysis of the same laboratory sample, lie within the range of the mean values cited in the table below, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility limit (*R*), which can generally be deduced by linear interpolation from the values in the table below.

APPENDIX 2. DISTRIBUTION OF POLAR COMPOUNDS IN OILS AND FATS BY HIGH-PERFORMANCE SIZE-EXCLUSION CHROMATOGRAPHY

1. Scope

This Standard describes a method to determine the contents of polymerized triacylglycerols, oxidized triacylglycerols, and diacylglycerols in oils and fats.

2. Field of application

This method is applicable to the polar fraction (obtained by Standard Method 2.507 or by that proposed in Appendix 1) of any kind of fats and oils, heated or not.

3. Principle

Dissolution of the sample in a suitable solvent and separation of the main groups of components by gel permeation chromatography according to molecular size (*Note 1*).

4. Apparatus

- 4.1 Solvent reservoir of about 250 mL of capacity with a mobile-phase liner filter (pore size 1 μ m)
- 4.2 HPLC pump, pulseless, with a flow of 0.7-1.5 mL/min.
- 4.3 Injection valve with a 10- μ L loop and a suitable syringe with a volume of 50–100 μ L (Rheodyne or Valco)
- 4.4 Stainless steel columns: two columns 300 mm in length, 7.7 mm internal diameter, packed with a high-performance spherical gel made of styrene-divinylbenzene co-polymer; diameter of the particles: 5 μ m; pore size: 100 Å and 500 Å. Columns are connected in series in the order 500 Å and 100 Å. The storage of such column must be achieved in toluene.
- 4.5 Detector: refractive index detector with a sensitivity at full scale at least 1×10^{-4} of refractive index
- 4.6 Recorder and/or integrator: to allow display and accurate quantification of the peak areas

5. Reagents

- 5.1 Tetrahydrofuran, analytical grade
- 5.2 Toluene, analytical grade

6. Procedure

- 6.1 Starting up HPLC equipment
It is advisable to follow carefully the manufacturer's recommendations. Switch on the system and pump tetrahydrofuran at a rate of 1 ml/min to purge the whole system up to the injection valve.

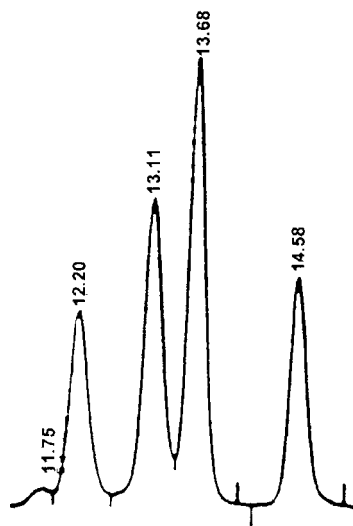


Fig. 3 Representative part of the HPSEC chromatogram. Retention times (min): 11.75 and 12.20, polymerized triacylglycerols; 13.11, oxidized triacylglycerols; 13.68, diacylglycerols and 14.58, fatty acids and polar unsaponifiable fraction.

Connect the column to the injection valve and wash it with about 30 mL of tetrahydrofuran. Connect the column to the sample cell of the detector. Fill the reference cell with the tetrahydrofuran. Adjust the mobile phase flow to 0.8–1.0 mL/min. Wait until a convenient stabilization of the system (no appreciable deviation of the baseline) is obtained (*Note 2*).

6.2 Preparation of the samples and analysis

Dissolve polar compounds (obtained by Standard Method 2.507 or by that proposed in Appendix 1) in tetrahydrofuran (5.1) at a concentration of 7–12 mg/mL. Take with the syringe 50–100 μ L of that solution. Fill the injection loop. Inject and switch on the integrator. With a mobile phase flow of 1 mL/min, the analysis time is about 20 min.

7. Results

7.1 Qualitative analysis

The chromatographic pattern of the determination normally shows five peaks as indicated in the typical chromatogram presented in Fig. 3. Eventually polymerized triglycerides may be absent and monoacylglycerols may appear between diacylglycerols and fatty acids (*Note 3*).

7.2 Quantitative analysis

The calculation is achieved assuming that all compounds of the sample are eluted.

The content of the different groups of compounds, expressed as percentage on oil, can be calculated as follows:

$$w_{\text{PTG}} = (A_{\text{PTG}} / \sum A) w_p$$

$$w_{\text{OTG}} = (A_{\text{OTG}} / \sum A) w_p$$

$$w_{\text{DG}} = (A_{\text{DG}} / \sum A) w_p$$

$$w_{\text{FA}} = (A_{\text{FA}} / \sum A) w_p$$

where

w_{PTG} is the percentage of polymerized triacylglycerols in the oil

w_{OTG} is the percentage of oxidized triacylglycerols in the oil

w_{DG} is the percentage of diacylglycerols in the oil

w_{FA} is the percentage of fatty acids in the oil, including the polar unsaponifiable matter

w_{p} is the percentage of polar compounds in the oil

A is the area of the corresponding peak

$\sum A$ is the sum of areas for all peaks

Some peaks may appear at longer retention time than that corresponding to fatty acids. They are normally due to residues and/or rests of solvents. Peak areas beyond that of fatty acids are excluded from calculations.

More than one peak corresponding to PTG may appear (11.75 and 12.20 min in Fig. 3). All peaks with shorter retention time than that of OTG must be summed up so as to be determined as a single group.

8. Quality assurance

- 8.1 For *general principles* of analytical quality control, see the section on Quality Assurance in the introductory part of the *Compendium of Standard Methods*.
- 8.2 For specific applications of analytical quality control, see the Annex to this standard method.

9. Notes

1. Polar compounds include groups of compounds differing in molecular weight formed through hydrolysis, oxidation, and polymerization reactions.
2. If the composition of the column is as indicated, an acceptable stabilization of the system should be obtained in about 15 min. With other column packing, the stabilization of the system may be more difficult: for example, changing the mobile phase should be done stepwise from toluene to tetrahydrofuran, with different mixtures, each time 25% higher in tetrahydrofuran. Acceptable stabilization is normally obtained in about 12 h.
3. In general, polymerized triglycerides are absent in crude or virgin oils, and low contents of monoacylglycerols are present in oils containing high amounts of diacylglycerols (i.e., palm oils).

Annex

1. *Repeatability*: When the mean of the values obtained from two single determinations carried out in rapid succession by the same operator, using the same apparatus under the same conditions for the analysis of the same laboratory sample, lies within the range of the mean values cited in the tables below, the difference between the two values obtained should not be greater than the repeatability limit (r), which can generally be deduced by linear interpolation from the values in the table below.

2. *Reproducibility*: When the values for the final result, obtained by operators in different laboratories using different apparatus under the same conditions for the analysis of the same laboratory sample, lie within the range of the mean values cited in the tables below, the difference between the values for the final result obtained by those operators should not be greater than the reproducibility limit (R), which can generally be deduced by linear interpolation from the values in the tables below.